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Parkin and the Molecular Pathways of Parkinson's Disease

Minireview

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Parkinson's disease (PD) is a neurodegenerative disease characterized by the selective demise of specific neuronal populations leading to impairment of motor functions. Recent genetic studies have uncovered several genes involved in inherited forms of the disease. These gene products are implicated in the biochemical pathways underlying the etiology of sporadic PD. Mutations in the *parkin* gene causal of autosomal recessive juvenile parkinsonism highlight that ubiquitin-mediated proteolysis may play an important role in the pathobiology of PD.

Introduction

Parkinson's disease (PD) is the most common movement disorder, with a prevalence of ~1% at 65 years of age but increasing to 4%–5% by the age of 85. It is characterized clinically by bradykinesia (slowed movement), resting tremor, rigidity, and postural instability. Although the neuronal circuitry involved in coordinated movement is complex, the disabling symptoms of PD are predominantly due to a profound reduction in striatal dopamine content caused by the demise of dopaminergic neurons in the substantial nigra (SN) pars compacta (Forno, 1996). The distinct pathological lesions of PD are round eosinophilic inclusions, comprised of a halo of radiating fibrils and a less-defined core, known as "Lewy bodies" (LBs), and dystrophic neurites termed "Lewy neurites" (LNs) (Forno, 1996).

The underlying cause(s) of most cases of PD is still unknown, but, recently, specific genetic defects have been identified in several kindred. Although this review focuses on these genes and their functions, we feel that it is also important to highlight that environmental factors, perhaps specific toxins, may contribute to the disease. In fact, Betarbet et al. recently reported that rats chronically challenged with rotenone, a plant-derived inhibitor of mitochondrial complex I that is used as a household pesticide and in the control of fish populations, develop the behavioral and histological features of PD (Betarbet et al., 2000). Furthermore, in both humans and animal models, oxidative stress is believed to be involved in the selective vulnerability of dopaminergic neurons of the SN due to their intrinsic predisposition to generate reactive species.

α-Synuclein Mutations and the Elucidation of the Composition of Lewy Pathology

The identification of a mutation (A53T) in the gene encoding the presynaptic protein α-synuclein (α-syn) in several kindreds with PD (Polymeropoulos et al., 1997) led to phenomenal progression in the characterization of Lewy pathology. α-syn is a small (140 amino acid) protein characterized by repetitive imperfect repeats (KTKEGV) distributed throughout most of the amino-terminal half of the polypeptide, a hydrophobic middle region (NAC region), and an acidic carboxy-terminal region (Figure 1A). The function of α-syn is not clearly established, but it appears to have some role in the modulation of synaptic vesicle turnover and synaptic plasticity (Clayton and George, 1998). α-syn has the ability to polymerize into ~10 nm fibrils in vitro, and bundles of these fibrils are the major components of LBs and LNs (Figure 1B). The A53T mutation in α-syn results from a G to A transition at position 209 and follows an autosomal dominant mode of transmission (Polymeropoulos et al., 1997). The pathological consequences of this mutation appear to stem from a significant increase in its propensity to polymerize. Furthermore, transgenic mouse and *Drosophila* models overexpressing α-syn suggest that the formation of α-syn inclusions can lead to neuronal dysfunction and other phenotypes reminiscent of human disorders (Dawson, 2000).

α-syn pathology is not restricted to PD but rather is a prominent feature of a spectrum of diseases termed "α-synucleinopathies" (Duda et al., 2000). In some of these diseases such as dementia with LBs and LB variant of Alzheimer's disease, Lewy pathology is broadly distributed in the cerebrum. α-syn aggregation can also occur in oligodendrocytes in the form of glial cytoplasmic inclusions, which are the major characteristic features of multiple system atrophy.

Mutations in the Parkin Gene Are Causal of Autosomal Recessive Juvenile Parkinsonism

Autosomal recessive juvenile parkinsonism (AR-JP) is a disease entity that has been shown to result from the loss of function of the *parkin* gene (Kitada et al., 1998). These patients display the typical clinical features of parkinsonism, but they also often present with foot dystonia, benefit from sleep, and marked response to L-dopa therapy. Onset of this disease can be as early as the first decade of life, but, for most cases, the onset is later than what could be considered "juvenile." In fact, onset is best described as before the age of 40, although some rare cases can have onset as late as the sixth decade of life (Klein et al., 2000). Neuropathological examination demonstrates that neuronal loss and gliosis are restricted to the SN and locus ceruleus (Hayashi et al., 2000; Mori et al., 1998). In several cases, Lewy pathology was not observed (Hayashi et al., 2000; Mori et al., 1998), leading to the speculation that the etiology of AR-JP differs significantly from PD. However, the recent autopsy of a patient with a 40 bp deletion in exon 3 in one allele of *parkin* and a R275W mutation in the other revealed the presence of Lewy pathology in the typical regions affected in PD (Farrer et al., 2001).

The *parkin* gene maps to chromosome 6q25.2-q27 and spans more than 500 kbp, and its 12 exons encode a 465 amino acid protein (Kitada et al., 1998). Parkin bears homology to ubiquitin at its N terminus, and two

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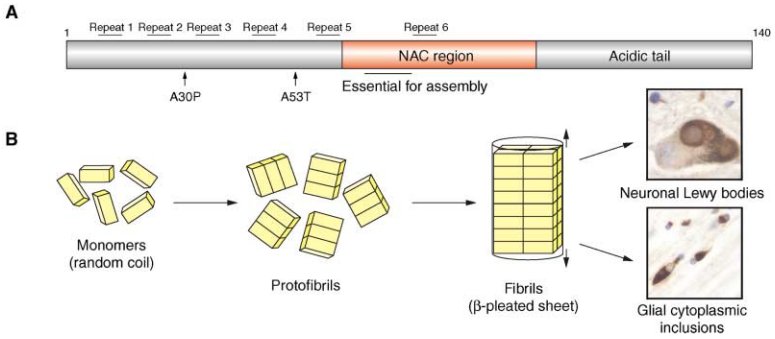

Figure 1. α-Synuclein Structure and Hypothetical Model for Its Aggregation

(A) Schematic representation of α-syn and the mutations associated with familial PD.
(B) Polymerization of α-syn resulting in the formation of neuronal or glial inclusions.

RING finger motifs are separated by an in-between RING fingers (IBR) domain near the C terminus (Figure 2). The most common parkin mutations are deletions of one or several exons, but duplications and triplications have also been observed (Kitada et al., 1998; Lucking et al., 2000). Some of these deletions are predicted to result in premature termination of translation due to frameshifts; however, the deletions of exon 5 or exon 3 plus exon 4 are in-frame deletions which are predicted to result in the expression of shorter proteins of 437 (deletion of codons 179 to 206) and 344 (deletion of codons 58 to 178) residues, respectively. Frameshift mutations resulting from the insertion or deletion of one, two, or five base pairs (Lucking et al., 2000), nonsense mutations, and missense mutations (Figure 2) are also causal to disease, but so far it appears that all mutations are recessive. Mutations in the *parkin* gene have been found on both alleles for the majority of patients with AR-JP. In some rare cases, mutations were identified in only one allele (Lucking et al., 2000), but there is likely an undetected complementary mutation in the other allele, perhaps in the promoter or in an intron. The cluster of mutations in the RING fingers and the IRB domain suggests that the preservation of these regions is important for function.

Parkin Is an E3 Ligase

Recent findings have shed new light on the function of RING finger proteins. Many of these proteins appear to have a critical role in mediating the ubiquitination of specific proteins, as well as themselves (Joazeiro and Weissman, 2000). Ubiquitin tagging of membrane-bound cytoplasmic and nuclear proteins identifies these molecules as a target for degradation by the proteasome and can also facilitate the endocytosis of plasma membrane proteins to lysosomes for degradation (Hershko and Ciechanover, 1998). Ubiquitin is a 76 amino acid protein that is usually convalently linked to the ϵ amino group of Lys residues in the targeted proteins by the formation of an isopeptide with its C terminus. Targeting to the proteasome usually requires the formation of a polyubiquitin chain, where the C terminus of each ubiquitin is added to a specific Lys residue of the previous ubiquitin. Ubiquitination is a tightly regulated process that requires at least three steps: (1) formation of an ATP-dependent thioester linkage between ubiquitin and the activating enzyme, E1; (2) transfer of the anchored ubiquitin moiety to a ubiquitin-carrier protein, E2; and (3) ubiquitination of the targeted protein by the E2/E3 complex. Target specificity is dictated by the functional interaction between E3 and the substrate.

Many RING finger proteins (Joazeiro and Weissman, 2000), including parkin (Shimura et al., 2000; Zhang et al., 2000), have been shown to act as E3 ligases. Parkin can interact with the E2 ubiquitin-carrier proteins UbcH7 and UbcH8, resulting in functional ubiquitination (Figure 3). Similar to other E3 RING finger proteins (Joazeiro and Weissman, 2000), the RING finger region of parkin confers the binding site for E2s (Imai et al., 2000; Shimura et al., 2000; Zhang et al., 2000). Mutations in parkin can

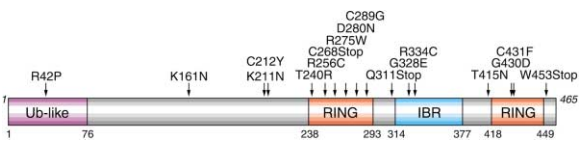

Figure 2. Schematic Representation of the Parkin Protein

Parkin consists of several domains: an amino-terminal ubiquitin-like domain (Ub-like) and two carboxy-terminal RING finger domains separated by an IBR. The location of disease-related point mutations resulting in amino acid substitutions or protein truncation is depicted. Additional frameshift and deletion mutants have been described; see text for details.

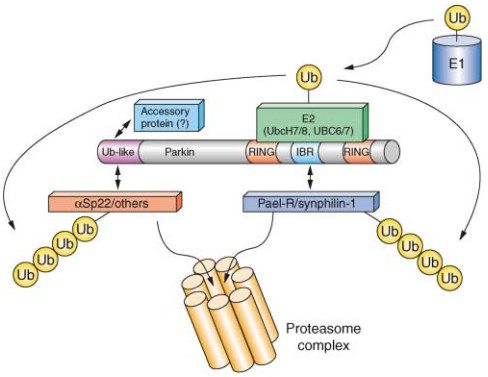

Figure 3. Involvement of Parkin in the Ubiquitin-Proteasome Pathway

Parkin (E3) serves as a link between the substrate for ubiquitination and E2s, which bind to the RING finger region. Alternate regions in parkin may recognize different substrates. The ubiquitin-like region is either required for the recruitment of an accessory protein or the binding of some substrates that may include αSp22. Pael-R and synphilin-1 interacts with the RING finger region, but synphilin-1 preferentially binds to the IBR region and the second RING finger domain. Ubiquitin moieties are transferred from E1 to E2 and finally to the substrate, which becomes a target for degradation by the proteasome.

disrupt the interaction with E2s, and it can also impair substrate recognition. The AR-JP-associated mutations Q311stop (resulting in a protein lacking the IRB and second RING finger) or T240R abrogate the interaction between parkin and UbcH7 (Shimura et al., 2000, 2001). The mutation R42P does not affect the interaction with UbcH7 but still abolishes ubiquitination, suggesting that it can disrupt the recruitment of a necessary accessory protein or the binding of some substrates (Shimura et al., 2000, 2001). Interaction with UbcH8 is eliminated by the Q311stop and T415N mutations, while the mutations T240R and W453stop reduce binding (Zhang et al., 2000). Seemingly at odds with the notion that the RING domain region is the E2 binding site, the in-frame exon 3-4 deletion, which results in the ablation of codons 58 to 178, prevents the interaction of parkin and UbcH7 or UbcH8 (Imai et al., 2000). These results remain unexplained, but it is possible that the omission of this stretch of residues results in a misfolded protein with a nonfunctional binding site. Nevertheless, these results also demonstrate that the exon 3-4 deletion mutant results in the loss of E3 ligase activity. Like other E3 ligases (Joazeiro and Weissman, 2000), parkin can induce its self-ubiquitination and degradation, and disease-associated mutations in the RING finger domains also can impair these properties (Imai et al., 2000; Zhang et al., 2000).

The activity of parkin as an E3 ligase and the impairment thereof by pathological mutations suggests that ubiquitination may be important in the etiology of PD. Consistent with this notion is the presence of ubiquitin moieties in all types of α-syn inclusions. Furthermore, in two siblings with parkinsonism, a substitution (I93M) was identified in ubiquitin carboxy-terminal hydrolase L1(UCHL-1), another enzyme involved in ubiquitin metabolism (Leroy et al., 1998). Since this amino acid change or other substitutions in UCHL-1 has not been identified in other families with PD, it is still not entirely clear if the I93M substitution is a dominant mutation causal of disease or an extremely rare variant that cosegregates with disease. This uncertainty notwithstanding, the mutation I93M in UCHL-1 results in a partial loss of catalytic activity (Leroy et al., 1998). UCHs constitute a large family of deubiquitinating enzymes that can cleave polymeric ubiquitin into monomers. These enzymes may reduce the ubiquitin-tagged degradation of specific proteins, and they can be important for the regeneration of free and reusable ubiquitin following protein degradation (Hershko and Ciechanover, 1998).

Parkin Substrates and Possible Disease Mechanisms

Zhang et al. demonstrated that parkin interacts with and promotes the ubiquitination of the synaptic vesicle-associated protein CDCrel-1 (Zhang et al., 2000). Parkin increases the degradation of CDCrel-1, while the mutations Q311stop and T415N do not affect turnover. CDCrel-1 may regulate the functioning of synaptic vesicles, such as transport and release, and it is possible that the increased abundance of this protein due to the impairment of parkin may reduce synaptic release, but this possibility remains to be evaluated.

Synphilin-1, which was identified and cloned as an α-syn-interacting protein, also is a substrate for parkin-targeted ubiquitination (Chung et al., 2001). The function of synphilin-1 is unknown, but it is present in LBs, and its coexpression with α-syn in cultured cells results in the formation of aggregates containing both proteins (Chung et al., 2001). In the presence of parkin, a significant percentage of these aggregates become ubiquitinated.

Parkin may also have a role in the regulation of α-syn metabolism. It can exist in complex with UbcH7 and a newly identified O-glycosylated isoform of α-syn (αSp22) that contains complex monosaccharide chains (Shimura et al., 2001). However, whether or not αSp22 is present in brain is controversial, since most laboratories have not been able to detect it, and this type of complex glycosylation is highly unusual for a cytoplasmic protein. The specificity of parkin for glycosylated isoform of α-syn is very stringent, as nonglycosylated α-syn is completely inert to parkin (Shimura et al., 2001; Chung et al., 2001). Wild-type parkin can promote the ubiquitination of αSp22, but the R42P and T240R mutants do not display this activity. Although the abundance of αSp22 is extremely scarce in normal brain, it is found at modest levels in AR-JP brains lacking parkin activity (Shimura et al., 2001). Shimara et al. proposed that ubiquitination of αSp22 may be a prerequisite in the formation of Lewy pathology, since it was believed that AR-JP patients do not have these inclusions. The recent report of an AR-JP patient with LBs and LNs (Farrer et al., 2001) demonstrates that some of these patients can develop these inclusions, but the presence of Lewy pathology may depend on the nature of the mutation. AR-JP patients with homologous null mutations appear to have no Lewy inclusions (Hayashi et al., 2000), while the patient described by Farrer et al. harbors a null mutation in one allele and a R275W mutation in the other. The latter mutation reduces the catalytic activity of parkin, but it still has substantial enzyme activity (Chung et al., 2001). Whether or not parkin is required for the formation of Lewy pathology remains speculative, and further pathological examinations of additional patients with parkin mutations will be required to resolve this issue.

We propose that Lewy pathology might contribute to neuronal cell death by the sequestration of functional parkin, which serves to degrade specific proteins. The inability to remove these proteins can eventually lead to toxicity. Indeed, evidence to support this notion includes the detection of parkin in LBs (Shimura et al., 2001) and its accumulation into inclusions comprised of α-syn and synphilin-1 in cultured cells (Chung et al., 2001). It is possible that the attempt of parkin to target these inclusions for degradation results in its loss of function. This model is consistent with the finding that patients with null mutations in parkin do not require Lewy pathology for disease, whereas Lewy pathology is present in the patient with the R275W mutation. Moreover, the reduction in parkin activity can contribute to inclusion formation, since synphilin-1, which may induce the formation of α-syn aggregates, is a substrate for parkin and therefore may accumulate in cells with reduced parkin activity.

A more diverse role of parkin is suggested by its increased expression following cellular stress associated with the accumulation of unfolded proteins (Imai et al., 2000). Parkin can assist in the removal of misfolded proteins in the endoplasmic reticulum (ER), as demonstrated for a homolog of endothelin receptor type B,

renamed "Pael-R" (Parkin-associated endothelin receptor-like receptor) (Imai et al., 2001). The ubiquitination and proteasome-mediated degradation of Pael-R can be modulated by parkin. Overexpression of parkin can suppress cell death associated with ER stress (Imai et al., 2000) and the accumulation of misfolded Pael-R, which can be toxic to cells (Imai et al., 2001). The ER-resident E2 proteins UBC6 and UBC7 act with parkin to target Pael-R for degradation. Consistent with this function of parkin, there is an increase in detergent-insoluble, presumably misfolded, Pael-R in the brains of AR-JP patients. Pael-R is widely expressed in the brain, predominantly in oligodendrocytes, but it is also expressed at exceptionally high levels in tyrosine hydroxylase-expressing neurons of the SN, perhaps contributing to the selective vulnerability of these neurons in AR-JP.

Dopaminergic nigral neurons may also be more vulnerable because of their intrinsic exposure to oxidative stress that may result in protein damage and misfolding. Parkin is widely expressed throughout the neuroaxis, and it is possible that it can also be sequestered into other types of pathological inclusions that share structural similarities to nigral LBs. The redistribution of parkin or other E3 ligases into proteinaceous inclusions may be a common pathological mechanism.

The identification and characterization of defective genes involved in the inherited forms of PD revealed some of the primary causes of these disorders, but they also provide insights into the biochemical pathways that may also be involved in the sporadic forms of the disease. The wide range in the age of presentation, even in some patients with the same gene defect, suggests that other contributors, either modulatory genes or environmental factors, are involved in the pathobiology of PD.

Selected Reading

Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., and Greenamyre, J.T. (2000). Nat. Neurosci. *3*, 1301–1306.

Chung, K.K.K., Zhang, Y., Lim, K.L., Tanaka, Y., Huang, H., Gao, J., Ross, C.A., Dawson, V.L., and Dawson, T.M. (2001). Nat. Med., in press.

Clayton, D.F., and George, J.M. (1998). Trends Neurosci. *21*, 249–254.

Dawson, V.L. (2000). Science *288*, 631–632.

Duda, J.E., Lee, V.M.-Y., and Trojanowski, J.Q. (2000). J. Neurosci. Res. *61*, 121–127.

Farrer, M., Chan, P., Chen, R., Tan, L., Lincoln, S., Hernandez, D., Forno, L.S., Gwinn-Hardy, K., Petrucelli, L., Hussey, J., et al. (2001). Ann. Neurol., in press.

Forno, L.S. (1996). J. Neuropathol. Exp. Neurol. *55*, 259–272.

Hayashi, S., Wakabayashi, K., Ishikawa, A., Nagai, H., Saito, M., Maruyama, M., Takahashi, T., Ozawa, T., Tsuji, S., and Takahashi, H. (2000). Mov. Disord. *15*, 884–888.

Hershko, A., and Ciechanover, A. (1998). Annu. Rev. Biochem. *67*, 425–479.

Imai, Y., Soda, M., and Takahashi, R. (2000). J. Biol. Chem. *275*, 35661–35664.

Imai, Y., Soda, M., Inoue, H., Hattori, N., Mizuno, Y., and Takahashi, R. (2001). Cell *105*, 891–902.

Joazeiro, C.A., and Weissman, A.M. (2000). Cell *102*, 549–552.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., and Shimizu, N. (1998). Nature *392*, 605–608.

Klein, C., Pramstaller, P.P., Kis, B., Page, C.C., Kann, M., Leung, J., Woodward, H., Castellan, C.C., Scherer, M., Vieregge, P., et al. (2000). Ann. Neurol. *48*, 65–71.

Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta, G., Brownstein, M.J., Jonnalagada, S., Chernova, T., et al. (1998). Nature *395*, 451–452.

Lucking, C.B., Durr, A., Bonifati, V., Vaughan, J., De Michele, G., Gasser, T., Harhangi, B.S., Meco, G., Denefle, P., Wood, N.W., et al. (2000). N. Engl. J. Med. *342*, 1560–1567.

Mori, H., Kondo, T., Yokochi, M., Matsumine, H., Nakagawa-Hattori, Y., Miyake, T., Suda, K., and Mizuno, Y. (1998). Neurology *51*, 890–892.

Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., et al. (1997). Science *276*, 2045–2047.

Shimura, H., Hattori, N., Kubo, S., Mizuno, Y., Asakawa, S., Minoshima, S., Shimizu, N., Iwai, K., Chiba, T., Tanaka, K., and Suzuki, T. (2000). Nat. Genet. *25*, 302–305.

Shimura, H., Schlossmacher, M.G., Hattori, N., Frosch, M.P., Trockenbacher, A., Schneider, R., Mizuno, Y., Kosik, K.S., and Selkoe, D.J. (2001). Science *293*, 263–269.

Zhang, Y., Gao, J., Chung, K.K., Huang, H., Dawson, V.L., and Dawson, T.M. (2000). Proc. Natl. Acad. Sci. USA *97*, 13354–13359.